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ABSENCE OF LATITUDINAL CLINES IN SPERM CHARACTERS IN NORTH AMERICAN POPULATIONS OF *DROSOPHILA SUBOBSCURA* (DIPTERA: DROSOPHILIDAE)

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Abstract.—Within the past twenty years, *Drosophila subobscura* (Collin 1936), endemic to the Old World, has rapidly colonized South America and the west coast of North America. Despite the recent colonization, inversion polymorphism and body size clines in North America have converged on clines found in Europe, indicating rapid evolutionary responses. One rapidly evolving trait in *Drosophila* is sperm length, which varies 180 fold between species. In species of the *obscura* group, to which *D. subobscura* belongs, sperm length also varies within individuals, a phenomenon known as sperm heteromorphism, in which males produce both short and long sperm. I examined sperm length evolution in males from eleven North American populations of *D. subobscura* representing a latitudinal transect of 1750 km. Significant differences between the populations in short and long sperm head lengths, and the total length of long sperm were found. However, these differences were not explained by latitude. A significant effect of males within a population on sperm length parameters was found indicating that sperm length variation within a population was equal to or exceeded variation across populations. Additionally, a potential constraint or stabilizing selection on sperm design was identified in that the ratio of short head to short tail compared to that ratio of long sperm did not differ. Results from this analysis suggest that significant inter-population variance in sperm length is unrelated to predictable environmental variation that mediates other traits, such as body size.

Key Words.—Insecta, Diptera, *Drosophila subobscura*, cline, sperm length, sperm heteromorphism.

Clines in genetic and morphometric traits, such as chromosomal inversions and body size, may result from historical processes and/or adaptive selection (Endler 1986). In a geographically wide-ranging species, such as some *Drosophila* species, the demonstration of parallel clines on different continents is taken as strong support for adaptive evolution (Endler 1986), presumably related to climate. In *Drosophila*, chromosomal polymorphisms (e.g., Anderson 1981, Oakeshott et al. 1982, Prevosti et al. 1985, 1988, Ayala et al. 1989, Berry & Kreitman 1993) and body size clines (e.g., Prevosti 1955, Coyne & Beecham 1987, Capy et al. 1993) have been well-documented and are thought to reflect climatic factors, such as temperature, rainfall and relative humidity (James et al. 1995).

Drosophila subobscura (Collin 1936) has recently expanded its geographic range, from its endemic Palearctic regions, to colonization and establishment of populations in both South (Brncic et al. 1981) and North America (Beckenbach & Prevosti 1986). Colonization in the New World occurred in the late 1970s and early 1980s and, since this accidental introduction, these populations have rapidly expanded along the western coasts of the South and North American continents such that, in certain areas, they can be the most abundant species (Ayala et al. 1989) and have been found as far west as Utah (Noor et al. 2000). Allozyme

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(Prevosti et al. 1983, Balayna et al. 1994), chromosomal (Prevosti et al. 1985, 1988; Ayala et al. 1989, Krimbas & Powell, 1992), and DNA (Latorre et al. 1986, Rozas et al. 1990, Rozas & Aguade 1991) polymorphisms have been studied in these newly founded populations. Significant latitudinal clines of chromosomal polymorphisms in both North and South America populations have been described that converge on clines found in Old World populations (Prevosti et al. 1990). Additionally, strengths for S. American chromosomal polymorphism clines increased between 1981 and 1986 (Prevosti et al. 1990), indicating the clines are rapidly evolving and are potentially adaptive. In addition to chromosomal clines, converging body size clines for both Old and New World *D. subobscura* populations have been documented (Pegueroles et al. 1995, Huey et al. 2000), indicating rapid evolution and possible selection for morphometric traits (Pegueroles et al. 1995).

A morphometric trait that appears to be rapidly evolving in *Drosophila* is sperm length (Joly et al. 1989, 1991; Pitnick et al. 1995). *Drosophila* exhibit the greatest inter-specific variation in sperm length (ranging from ca. 0.090 to 58 mm; Pitnick et al. 1995) of any animal taxa so far examined. The adaptive significance of sperm length is under debate (Birkhead & Møller, 1998), but is likely related to sexual selection (e.g., Pitnick et al. 1995, Pitnick & Miller 2000). Additionally, in a phylogenetically controlled study, Pitnick and colleagues (1995) found that on a macroevolutionary scale, species in which males were bigger produced longer sperm. Given that several *Drosophila* species, including *D. subobscura*, exhibit body size clines, that the body size cline in *D. subobscura* occurred rapidly and that *Drosophila* sperm length is a rapidly evolving trait, the question of whether intra-specific clinal variation in *D. subobscura* sperm length exists is an intriguing one.

Members of the *obscura* group, including *D. subobscura* and *D. pseudoobscura* (Frolowa 1929), are also of particular interest regarding sperm length evolution because males exhibit sperm heteromorphism in which they simultaneously produce two lengths of sperm, short and long (Fig. 1; Beatty & Sidhu 1970, Snook et al. 1994, Bressac & Hauschteck-Jungen 1996, Snook 1997). Sperm heteromorphic species produce and transfer to females both sperm types, and females store both sperm types, at least transiently (Beatty & Sidhu 1970, Snook et al. 1994, Bressac & Hauschteck-Jungen 1996). However, the different sperm lengths are functionally nonequivalent in that only long sperm participate in fertilization (Snook et al. 1994, Snook & Karr 1998). The evolutionary function of short sperm in the *obscura* group remains a conundrum (Snook 1998a).

Here I examine sperm length evolution in eleven populations of North American *D. subobscura*, representing a latitudinal transect across 15° and 1750 km. I determine whether these populations exhibit nonrandom geographic variation (i.e., cline) in sperm length parameters (head, tail and total lengths) of both short and long sperm. I also examine the evolution of these sperm length parameters within a sperm type and identify a potential constraint on sperm design.

Methods and Materials

Flies.—Populations of *D. subobscura* from western North America were collected in 1997 by R. Huey and colleagues (Huey et al. 2000). They collected flies on yeasted banana baits at eleven sites at 1.5° intervals between Atascadero, Cal-

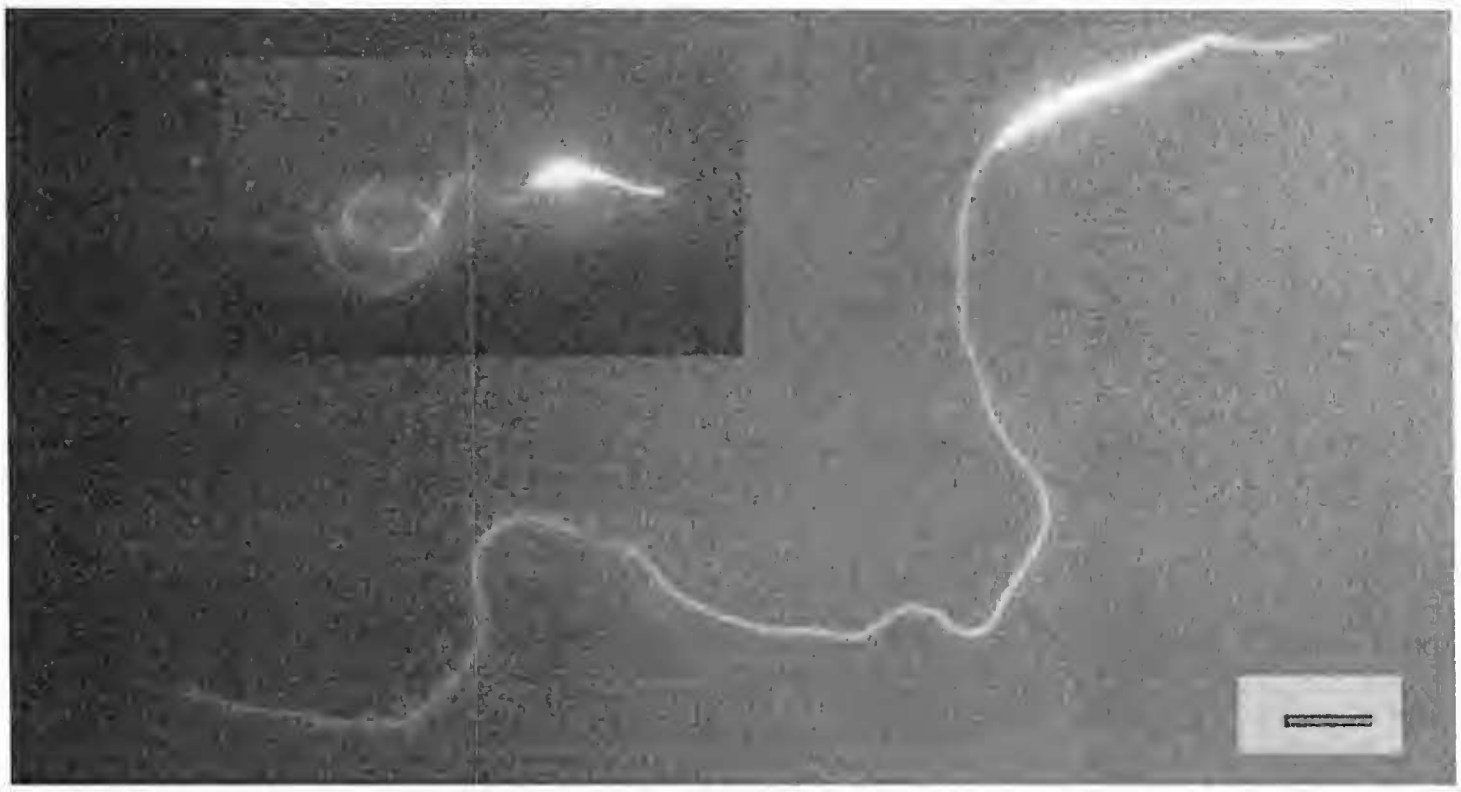

Figure 1. Dimorphic sperm from the seminal vesicles of *D. subobscura.* Sperm were dissected, processed and stained with a DNA-specific fluorescent dye as described previously (Snook et al. 1994). Inset: short sperm type. Scale bar = 25 μm.

ifornia and Port Hardy, Canada (Fig. 2) and subsequently established isofemale lines of *D. subobscura.* To form populations, 10 F1 males and 10 F1 females from 25 isofemale lines per locality were combined. Once I received the flies, they were reared in vials containing yeasted banana food at ca. 22° C. Flies went through less than 20 generations from the time of collection to this study.

Sperm Length Measurements.—To measure sperm, virgin males were collected by aspiration upon eclosion and stored in 25 mm × 95 mm food vials until males were 7 days old. Each male was ether-anaestetized and dissected in phosphate buffer solution to remove the reproductive tract. Sperm were removed from the seminal vesicles and processed for sperm length measurements on gelatin/chrome alum-coated glass microscope slides as previously described (Snook et al. 1994).

Computer images of sperm were taken using a Zeiss AxioPlan epifluorescent microscope and a Princeton Instruments Quantum camera. Sperm heads and tails were measured using the length measurement function in IP Lab Spectrum. The total lengths of sperm were obtained by adding the head and tail length for each sperm measured. From 15 to 10 sperm of each sperm type from 3 or 4 males in each population were measured (Table 1).

Statistics.—To test if populations significantly differed in sperm length parameters, nested ANOVAs were performed with males nested within a population. A potential ecological determinant of sperm length through latitudinal and body size clines for sperm lengths were assessed by linear regression. The relationship of the ratio between short head and tail lengths compared to that ratio in long sperm was tested using a paired t-test. All statistics were performed with JMP (SAS 1995).

RESULTS

Sperm length data for each male were normally distributed. Means and standard error of the mean for each sperm length parameter in all populations are reported

Figure 2. Collection sites of *D. subobscura* along the west coast of North America.

in Table 1. Nested ANOVAs indicated a significant effect of male nested within population for each sperm length parameter (Table 2). Nested ANOVAs also indicated a significant difference between populations in short and long sperm head lengths and the total length of long sperm (Table 2). However, these differences were not correlated with latitude for any sperm length parameter (Table 3; Fig. 3).

In *obscura* group species, the length of sperm heads for both sperm types are notably longer (Fig. 1) than the more commonly studied *D. melanogaster* (Meigen 1830) or *D. hydei* (Sturtevant 1921) (Fuller 1993). In other *Drosophila* species, the primary variation in sperm length between species is the sperm tail. In *obscura* group species, head length comprises a larger ratio of the total length of the sperm (Snook 1997). I used a paired *t*-test to test the relationship between the ratio of sperm head to tail lengths, comparing short and long sperm types. This analysis revealed that short and long sperm have the same ratio of head: tail lengths ($t = 1.184$, $df = 36$, $P = 0.244$; Fig. 4) and that there was a significant positive relationship between the ratios (Fig. 4; $F = 15.6$, $df = 1, 35$, $P < 0.001$).

Table 1. The names of the eleven strains and their latitude, arranged from south to north, from which sperm morphometrics (mean ± SE) were determined. Sample sizes are below the means; the first number is the number of males and the second number is the total number of sperm measured per population.

Population	Lat (°N)	Sperm Morphometrics					
		Short Sperm			Long Sperm		
		Head	Tail	Total	Head	Tail	Total
Atasca	35.5	24.1 ± 0.7 4,60	103.3 ± 2.4 4,58	127.3 ± 2.9 4,58	53.0 ± 1.1 4,60	243.7 ± 4.8 4,60	296.7 ± 5.0 4,60
Gilroy	37.00	26.1 ± 0.6 3,30	105.7 ± 2.5 3,30	131.8 ± 2.7 3,30	54.3 ± 1.2 3,30	239.0 ± 6.2 3,30	293.3 ± 7.2 3,30
Davis	38.60	15.8 ± 0.5 3,30	105.2 ± 3.2 3,30	121.0 ± 3.3 3,30	33.9 ± 1.2 3,30	191.7 ± 4.5 3,30	225.7 ± 5.3 3,30
Redding	40.60	19.3 ± 0.6 3,24	93.6 ± 2.5 3,24	113.0 ± 2.5 3,24	47.1 ± 1.2 3,30	220.0 ± 4.7 3,30	267.2 ± 4.7 3,30
Eureka	40.80	22.7 ± 0.7 3,30	100.1 ± 2.5 3,30	112.8 ± 2.7 3,30	46.8 ± 1.8 3,30	242.1 ± 6.9 3,30	288.9 ± 8.1 3,30
Medford	42.30	19.5 ± 0.7 3,30	99.1 ± 2.7 3,30	118.6 ± 2.7 3,30	39.3 ± 0.6 3,30	228.6 ± 5.9 3,30	267.8 ± 5.9 3,30
Salem	44.90	23.7 ± 1.1 3,30	114.4 ± 2.2 3,30	138.1 ± 2.7 3,30	52.7 ± 1.0 3,30	265.4 ± 5.4 3,30	318.1 ± 5.9 3,30
Centralia	46.70	20.6 ± 0.5 4,60	115.5 ± 1.4 4,57	135.9 ± 1.3 4,57	44.3 ± 1.0 4,60	242.7 ± 2.8 4,59	286.7 ± 3.2 4,59
Bellingham	48.70	21.4 ± 0.7 3,30	105.8 ± 2.1 3,30	127.2 ± 2.2 3,30	46.7 ± 1.2 3,30	215.1 ± 5.0 3,30	261.8 ± 5.6 3,30
Peachland	49.80	24.7 ± 0.5 4,60	113.9 ± 1.4 4,58	138.6 ± 1.3 4,58	49.2 ± 0.9 4,60	241.4 ± 4.7 4,60	290.6 ± 5.1 4,60
Port Hardy	50.70	22.7 ± 0.5 4,60	105.2 ± 2.2 4,60	128.0 ± 2.3 4,60	48.1 ± 0.8 4,60	231.7 ± 4.3 4,60	279.8 ± 4.4 4,60
Overall		21.9 ± 0.9	105.6 ± 2.1	127.5 ± 2.5	46.9 ± 1.8	232.8 ± 5.8	279.7 ± 7.2

Table 2. Results from nested ANOVAs testing for differences between populations in sperm parameters with the effects of individual males from each population nested within population (Male (pop.).

Sperm Parameter	Source	SS	MS	df	F	P
Short Head	Population	2997.8	299.78	10	2.76	0.018
	Male (pop)	2982.08	114.69	26	10.95	0.0001
Short Tail	Population	16933.5	1693.35	10	1.47	0.21
	Male (pop)	31554.9	1213.26	26	8.89	0.0001
Short Total	Population	23236.7	2323.67	10	1.74	0.12
	Male (pop)	36527	1404.89	26	8.71	0.0001
Long Head	Population	12383.4	1238.34	10	3.07	0.01
	Male (pop)	10962.6	421.64	26	15.08	0.0001
Long Tail	Population	116973	11697.3	10	1.65	0.15
	Male (pop)	192665	7410.2	26	12.14	0.0001
Long Total	Population	183514	18351.4	10	2.25	0.0471
	Male (pop)	221629	8524.19	26	11.8	0.0001

Discussion

Several *Drosophila* species exhibit body size clines perhaps associated either with temperature, rainfall or relative humidity (James et al. 1995). The demonstration by Huey and colleagues (2000) that rapid clines in male and female body size were established in these *D. subobscura* populations suggests strong selection by some environmental component. Sperm length is a rapidly diverging trait in *Drosophila*, thus I addressed whether clinal variation in sperm length existed. While significant differences between populations in the lengths of short and long heads and in the total length of long sperm were identified, these differences were unrelated to latitude. Similarly, palearctic *D. subobscura* do not vary clinally in the number of teeth on sex combs and claspers (Perguoles et al. 1995). Interspecific variation in the meristic characters of sex comb teeth and claspers may play a role in sexual selection and influence isolating mechanisms, whereas intraspecific variation in these traits may not have an adaptive function and are used as taxonomic traits in *obscura* group phylogenies (Buzzati-Traverso & Scossironi 1955, Perguoles et al. 1995). Likewise, inter-specific variation in *Drosophila* sperm length may play a role in isolating processes (Joly et al. 1997, Snook 1997), whereas the adaptive function of intra-specific sperm length variation is debated (e.g., Birkhead & Møller, 1998).

In mice (Beatty 1970) and other insect systems (Gage & Cook 1994, Pitnick et al. 1995), sperm morphometric traits appear to be genetically determined (Beatty 1970), with minimal variability in sperm dimensions due to environmental effects (Beatty 1970, Gage & Cook 1994, Pitnick et al. 1995). In *Drosophila*, little work has been done examining either ecological or genetic factors controlling sperm length (Pitnick et al. 1995, Joly et al. 1997, Snook 1998b). Here I found that predictable changes in the environment associated with latitude do not influence alterations in sperm length, although significant intra-specific differences in sperm length existed. Intra-specific differences in sperm length in the *D. subobscura* populations studied may not be the result of selection but of drift due to the potentially small numbers of flies colonizing a particular area (Noor et al. 2000). The origin and number of emigrants colonizing these populations, in ad-

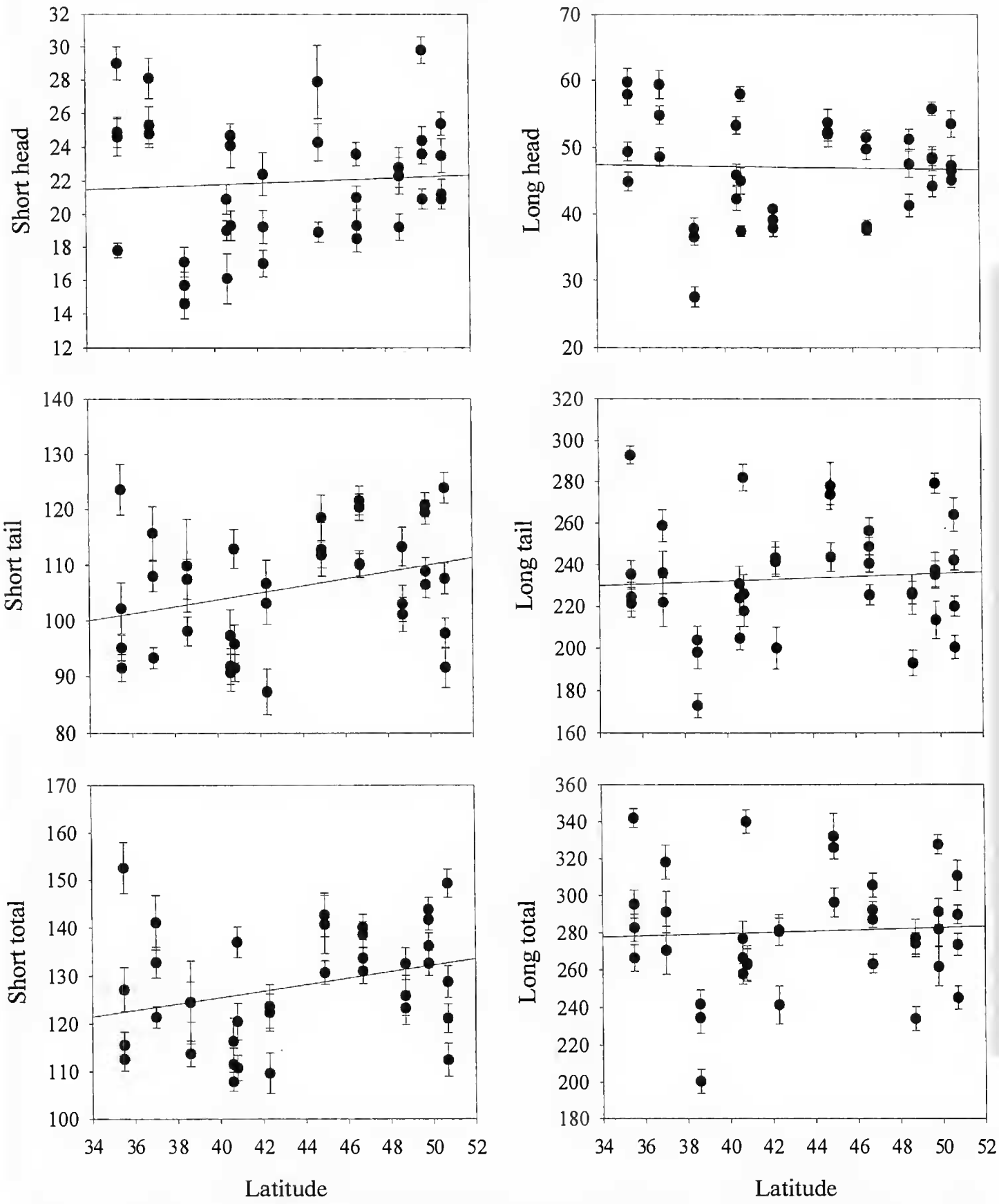

Figure 3. Relationships between sperm length parameters (μm) and latitude. See text for statistics.

dition to migration, are unknown. However, if drift is responsible for the population differences in sperm length, there was no corresponding effect on male and female body size given the rapid establishment of a latitudinal cline in body size in these same populations (Huey et al. 2000).

Nested ANOVA analyses revealed significant intra-population variation in sperm length parameters for all morphometrics, indicating that variance in sperm length parameters between males within a population is equal to or higher than variance between populations. The biological relevance of the high intra-population variance is unknown, but could be related to the outcome of sperm com-

Table 3. Correlations between sperm parameters and latitude for eleven *D. subobscura* populations.

Sperm Parameter	Latitude		
	R^2	F	P
Short Head	0.004	0.1494	0.70
Short Tail	0.09	3.77	0.06
Short Total	0.09	3.43	0.07
Long Head	0.001	0.03	0.86
Long Tail	0.005	0.17	0.68
Long Total	0.002	0.099	0.75

petition. In *Drosophila*, sperm are stored by females in two sperm storage organ types, paired mushroom-shaped spermathecae and a tubular ventral receptacle. Sperm length is highly correlated to ventral receptacle length, suggesting that sperm competitive ability or cryptic female choice may be mediated by the correspondence between sperm length and sperm storage length (Pitnick et al. 1999). If female receptacle length also varied significantly within populations, then selection pressures associated with sperm competition may alter sperm length in a manner consistent with ventral receptacle length. However, a recent analysis by Pitnick and Miller (2000) found that selection for increased sperm length in *D. melanogaster* did not result in a correlated increase in ventral receptacle length. Variation in sperm length may not be directly related to any parameter associated

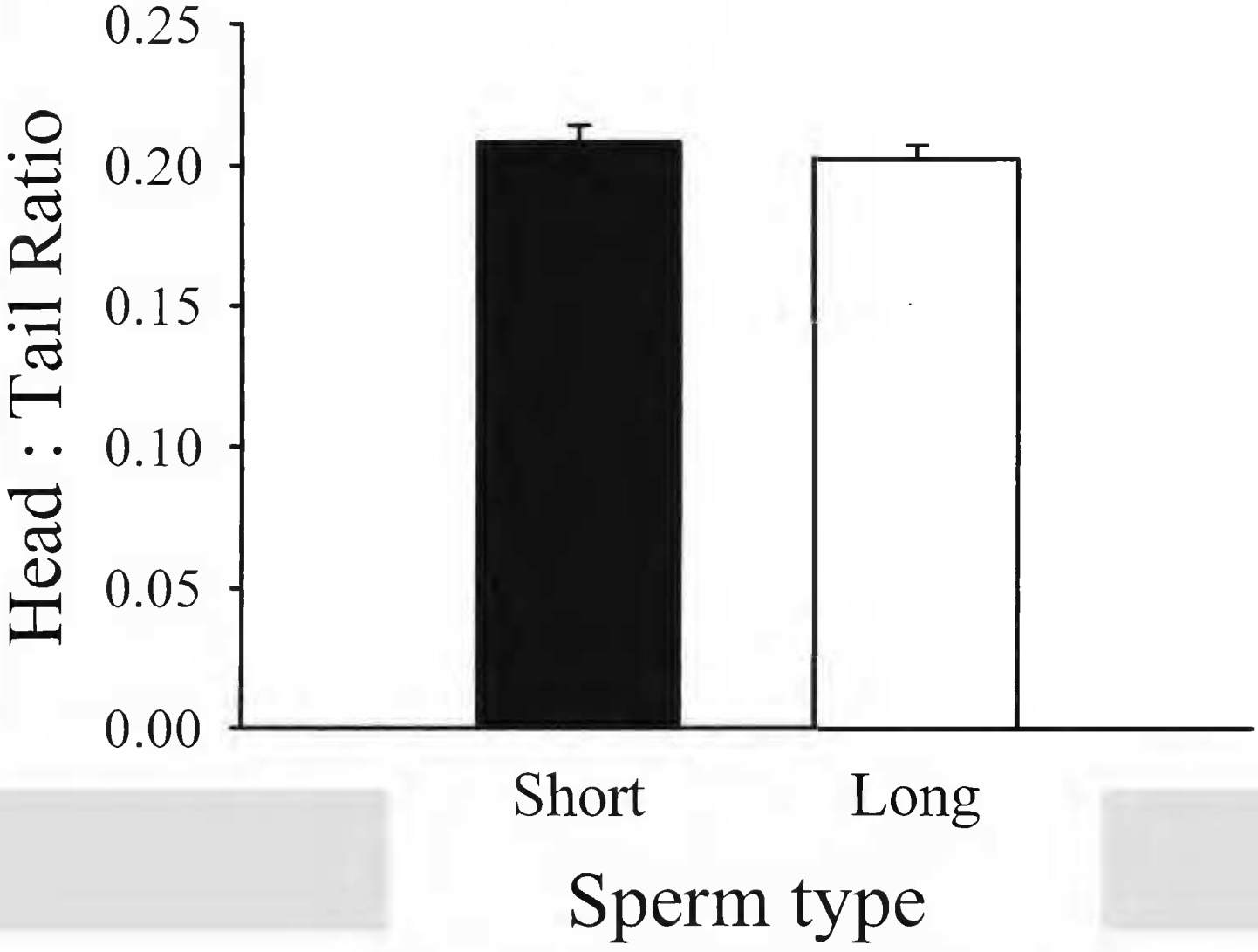

Figure 4. Relationship between the ratio of short head to short tail (μm) vs. the ratio of long head to long tail (μm). See text for statistics.

with latitude (e.g., temperature, rainfall) but with operational sex ratios and the risk of sperm competition. Unfortunately, these associations cannot be tested with the flies examined in this study.

Within a species, sperm length also does not seem to respond to male body size differences. Among the eleven *D. subobscura* populations that exhibit both male and female body size clines (Huey et al. 2000), I found no correspondence between sperm length and previously identified body size clines. Similarly, a recent study across sperm dimorphic diopsid flies found that male body size was unrelated to both short and long sperm lengths (Presgraves et al. 1999) and a lack of intra-specific association between sperm length and male body size was found in a single population of *D. hydei* (Pitnick & Markow 1994). In toto, these results indicate that factors influencing inter-specific sperm length variation, such as body size (Pitnick et al. 1995), are not similar to those influencing intra-specific variation.

Both short and long sperm had similar ratios in head compared to tail lengths regardless of the population from which males derived. This similarity suggests that there is either some isometric growth of sperm or a developmental constraint on sperm design. Alternatively, sperm design may be under stabilizing selection. In a phylogenetic analysis examining the evolution of sperm heteromorphism in the *obscura* group, long fertilizing sperm were found to be constrained by phylogeny, whereas short sperm were uncoupled from phylogeny and fertilization requirements, indicating independent evolutionary change (Snook 1999). Similarly, in sperm heteromorphic diopsid flies, the total length of short and long sperm were unrelated and suggests different selection pressures on sperm length evolution (Presgraves et al. 1999). These phylogenetic analyses examined total sperm length and not the effect of phylogeny on head and tail lengths. Given the consistent results between the sperm heteromorphic systems, however, it seems unlikely that the similar ratios of short and long sperm in *D. subobscura* are a result of stabilizing selection. Each sperm type serves a different function and thus selection should be acting differently on each type. Long sperm function in fertilization (Snook et al. 1994, Snook & Karr 1998) whereas the adaptive significance of short sperm remains a conundrum (Snook & Markow 1996, Snook 1998a). Thus, the similar ratios of head to tail lengths in both short and long sperm support the interpretation of either isometric growth or developmental constraint.

In conclusion, while chromosomal polymorphism and morphometric body size clines have quickly developed in recent New World populations of *D. subobscura* and while sperm length is a rapidly evolving trait in *Drosophila*, a sperm length cline has not developed. Furthermore, similarities in sperm design were identified that may indicate either proximate or ultimate mechanisms controlling sperm morphometrics. The intra-specific variation in sperm characteristics found in this study and others (Ward 1998) suggests extended work on genetic and ecological factors influencing sperm to more fully understand the evolutionary significance of this variation.

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